



The role of central melanocortin receptors in the activation of the hypothalamus-pituitary-adrenal-axis and the induction of excessive grooming

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1 In accord with previous studies intracerebroventricular (i.c.v.) injections of ACTH₁₋₂₄ (1 µg) induced a display of excessive grooming, and increased the plasma concentrations of ACTH and corticosterone. Pituitary-adrenal activation was blocked by pretreatment with dexamethasone, indicating that the effect of the (i.c.v.) injected peptide was not caused by a peripheral effect on the adrenal cortex.

2 Doses of 1 and 3 µg of a non-selective melanocortin-3/4-receptor antagonist (SHU 9119), or of 5 and 10 µg of a selective melanocortin-4-receptor antagonist ([D-Arg⁸]ACTH₄₋₁₀), coadministered (i.c.v.) with 1 µg ACTH₁₋₂₄, inhibited the ACTH₁₋₂₄-induced activation of the hypothalamus-pituitary-adrenal-axis and excessive grooming.

3 In addition, several doses of the selective melanocortin-3-receptor agonist Lys-γ₂-MSH were centrally administered, but neither neuroendocrine, nor excessive grooming responses were observed.

4 These results imply that the melanocortin-4-receptor, and not the melanocortin-3-receptor, is involved in the ACTH₁₋₂₄-induced rise in plasma levels of ACTH and corticosterone, and excessive grooming.

Keywords: Melanocortin-4-receptor; melanocortin-3-receptor; melanocortin receptor antagonist; ACTH; corticosterone; grooming

Introduction

The multifactorial precursor molecule pro-opiomelanocortin (POMC) is expressed in the anterior lobe of the pituitary gland, in certain peripheral tissues, and in restricted areas of the brain. Differential enzymatic processing of POMC in these tissues yields biologically active peptide end-products. Thus, POMC is the precursor of a large family of melanocortins, including adrenocorticotrophic hormone (ACTH), α-melanocyte stimulating hormone (α-MSH), β-MSH and γ-MSH, that function as hormones in the periphery and as neuromodulators in the brain. As such, the melanocortins play an important role in the regulation of an array of adaptive behavioural and physiological processes (De Wied & Jolles, 1982; Eberle, 1988).

We have previously shown, that intracerebroventricular (i.c.v.) administration of melanocortins induces excessive grooming behaviour in rats (Gispen *et al.*, 1975; Gispen & Isaacson, 1986), and in addition activates the hypothalamus-pituitary-adrenal-(HPA-) axis (Wiegant *et al.*, 1979). These studies revealed that these effects are both independent of corticosteroidogenic activity of the peptides. In addition, these effects are probably brought about via interaction of the peptides with different targets in the brain, since a reduced effectiveness of a second i.c.v. injection of ACTH (short-term tolerance) was found for the grooming-response, but not for the activation of the HPA-axis (Wiegant *et al.*, 1979).

Recently, the primary structure of the melanocortin (MC) receptor has been established by sequencing of cloned cDNA, and five types of receptor (MC₁ through to MC₅) have now been distinguished that differ with respect to their anatomical distribution and ligand selectivity. This heterogeneity probably underlies the diversity in biological actions of the melanocortin

peptide family (Tatro, 1990; Florijn *et al.*, 1993; Tatro, 1993; Adan *et al.*, 1994a; Low *et al.*, 1994; Tatro & Entwistle, 1994).

Expression of the MC₁- and the MC₂-receptors seems to be restricted to peripheral tissues, where the MC₁-receptor on melanotrophs mediates MSH stimulated pigmentation (Chhajlani & Wikberg, 1992), and the MC₂-receptor on adrenocortical cells mediates ACTH-induced steroidogenesis (Mountjoy *et al.*, 1992). In the brain, the receptors expressed are predominantly of the MC₃- and MC₄- types (Low *et al.*, 1994), although very low levels of MC₅-receptor mRNA have also been detected (Griffon *et al.*, 1994; Labbé *et al.*, 1994). MC₃-receptor mRNA is primarily found in the hypothalamus (Chhajlani *et al.*, 1993; Gantz *et al.*, 1993a; Roselli-Rehffuss *et al.*, 1993), whereas MC₄-receptor mRNA is expressed in a wide variety of regions throughout the brain (Gantz *et al.*, 1993b; Mountjoy *et al.*, 1994). The anatomical distribution of these central receptors is consistent with a role in the regulation of neuroendocrine, autonomic and behavioural responses.

In vitro studies with membrane binding assays and expression systems have shown that α-MSH, ACTH and γ-MSH-peptides are the most potent naturally occurring agonists for the MC₃-receptor (Chhajlani *et al.*, 1993; Gantz *et al.*, 1993a; Roselli-Rehffuss *et al.*, 1993; Low *et al.*, 1994). The MC₄-receptor is activated by ACTH and α-MSH, but not by γ₂-MSH (Gantz *et al.*, 1993b; Low *et al.*, 1994; Mountjoy *et al.*, 1994). For the MC₅-receptor, α-MSH and ACTH₁₋₂₄ are the agonists with highest potency and affinity, whereas γ₂-MSH is a weak, low affinity agonist for this receptor (Griffon *et al.*, 1994; Labbé *et al.*, 1994). Thus, the MC₃-receptor is the only known melanocortin receptor in the brain that can be activated by physiological levels of γ₂-MSH.

Recently, we have shown that melanocortin-induced excessive grooming is mediated by the MC₄-receptor in the brain (Adan *et al.*, 1994b). In view of our previous findings

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that induction of excessive grooming and activation of the HPA-axis following i.c.v. administration of melanocortins involve different mechanisms (Wiegant *et al.*, 1979), it was investigated whether or not different melanocortin receptors underly these effects. Neither selective antagonists for the MC₃-receptor, nor selective agonists for the MC₄-receptor are available at present. Therefore, a non-selective antagonist for the MC₃-receptor and the MC₄-receptor, that retains agonistic activity for the MC₅-receptor (Hruby *et al.*, 1995), and a selective antagonist for the MC₄-receptor (Adan *et al.*, 1994b) were tested in rats for their ability to antagonize the plasma ACTH and corticosterone response and the display of excessive grooming behaviour following i.c.v. injection of ACTH₁₋₂₄. In addition, a selective agonist for the MC₃-receptor, Lys-γ₂-MSH, was tested (i.c.v.) for its ability to stimulate HPA-activity and evoke excessive grooming.

Methods

Animals

Male Wistar rats (GDL; Utrecht, The Netherlands) of approximately 130 g were housed individually under standard laboratory conditions (21 ± 1°C; 60% humidity; lights on from 7 h 00 min until 19 h 00 min) and received food and water *ad libitum*. The experiments were performed between 10 h 00 min and 12 h 00 min. Experimental procedures were approved by the Committee on Animal Experiments of the Faculty of Medicine, Utrecht University.

Surgery, treatment, and assay procedure

I.c.v. injections of the peptides and/or antagonists were given in a volume of 3 μl saline. When coadministered, the peptide and antagonist were injected as a mixture. For i.c.v. injections, the brain ventricular system of the rats was cannulated as previously described (Brakkee *et al.*, 1979). The rats were anaesthetized by intramuscular injection of 1.0 ml kg⁻¹ bodyweight Hypnorm (fentanyl citrate 0.135 mg ml⁻¹ and fluanisone 10 mg ml⁻¹, Janssen Pharmaceutica, Beerse, Belgium). During the recovery period (at least ten days) the animals were frequently weighed, handled and trained to avoid handling-induced stimulation of the HPA-axis. The rats were transported to a low noise room at least three days before the experiments were performed. Injections were given by hand with a modified 10 μl Hamilton syringe.

The occurrence of grooming (Gispén & Isaacson, 1986) was recorded in the home cage with a 15th s time sampling procedure as described by Gispén *et al.* (1975). Since we expected the neuroendocrine response to reach a maximum at 30 min after i.c.v. application of ACTH (Wiegant *et al.*, 1979),

grooming was scored for 29 min starting from 1 min and continued until 30 min after the i.c.v. injection.

Immediately thereafter, the animals were killed by decapitation in an adjacent room, and trunk blood was collected in ice-cold 10 ml tubes containing anti-coagulant (15 i.u. heparin ml⁻¹ blood; Sarstedt, Nümbrecht, Germany). Separate control groups of saline- and ACTH₁₋₂₄-treated rats were run for each dose of a(nta)gonist.

Dexamethasone sodium-phosphate (Organon, Oss, The Netherlands) was used to block pituitary ACTH release in order to demonstrate that i.c.v. administration of ACTH₁₋₂₄ induced the neuroendocrine response at the level of the brain and not by leakage to the periphery (Keller-Wood & Dallman, 1984). To that end, dexamethasone (dissolved in Tris buffer; pH = 7.7) was given at a dose of 75 μg/rat s.c. twice, at 2 and 17 h before the experiment.

ACTH and corticosterone radioimmunoassays

Plasma ACTH was determined in duplicate by direct radioimmunoassay (RIA) according to the procedure described by Van Oers *et al.* (1991), by use of a specific rabbit antiserum, directed to the mid-portion of ACTH (code Ft 8514) kindly donated by Dr G.B. Makara (Budapest, Hungary). The antiserum did not cross-react with [D-Arg⁸]ACTH₄₋₁₀, SHU 9119, Lys-γ₂-MSH and dexamethasone sodium-phosphate. Cross-reactivity for ACTH₁₋₂₄ was 400%. Synthetic human ACTH₁₋₃₉ (Peninsula Laboratories, Belmont, CA, U.S.A.) was used as standard, and ¹²⁵I-labelled ACTH₁₋₃₉ (Iodogen method) as tracer. Sample dilution curves paralleled the standard curve. The sensitivity of the assay, calculated at B/B₀ = 0.9, was 10 pg ml⁻¹ plasma (0.5 pg/tube). Intra- and interassay variations were 5% and 8%, respectively.

Plasma corticosterone was determined in triplicate after dichloromethane extraction (recovery approximately 100%) by RIA according to the procedure described by Van Oers *et al.* (1991). A specific sheep antiserum, code S-052305676, kindly donated by Dr T.J. Benraad (Nijmegen, The Netherlands) was used. The antiserum hardly cross-reacted with [D-Arg⁸]ACTH₄₋₁₀ (<0.2%), SHU 9119 (<0.2%), Lys-γ₂-MSH (<0.1%), ACTH₁₋₂₄ (<0.2%) and dexamethasone sodium phosphate (<0.1%). Corticosterone (Sigma Chemical Co., St. Louis, MO, U.S.A.) was used as a standard and ³H-labelled corticosterone as tracer. Sample dilution curves paralleled the standard curve. The sensitivity of the assay, calculated at B/B₀ = 0.9, was 0.4 ng ml⁻¹ plasma (20 pg/tube). Intra- and interassay variations were 4% and 7%, respectively.

Peptides and antagonists

ACTH₁₋₂₄ and [D-Arg⁸]ACTH₄₋₁₀ (Table 1) were obtained by Organon (Oss, The Netherlands). The selective MC3-R

Table 1 The amino acid sequences* of the peptides and antagonists used

ACTH ₁₋₂₄	H-Ser-Tyr- Met-Glu-His-Phe-Arg-Trp-Gly -Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-OH (non-selective MC ₃ /MC ₄ receptor agonist)
SHU 9119	Ac-Nle-Asp- His -D-Nal(2)- Arg-Trp -Lys-NH ₂ ** (non-selective MC ₃ /MC ₄ receptor antagonist; MC ₅ -receptor agonist)
[D-Arg ⁸]ACTH ₄₋₁₀	H- Met-Glu-His-Phe -D-Arg- Trp-Gly -OH (selective MC ₄ -receptor antagonist)
Lys-γ ₂ -MSH	H-Lys-Tyr-Val- Met -Gly- His-Phe-Arg-Trp -Asp-Arg-Phe-Gly-OH (selective MC ₄ -receptor agonist)

*Bold and underlined: original amino acids in the ACTH₄₋₁₀ sequence. **Lactam bridge between Asp and Lys.

agonist, Lys- γ_2 -MSH (Table 1) was synthesized and kindly donated by Dr P. Hoogerhour (RIVM, Utrecht, The Netherlands). SHU 9119 (Table 1) was a gift from Dr R. D. Cone (Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, U.S.A.). Peptides and antagonists were dissolved in saline.

Statistics

Statistical analysis of data was performed by an one-way ANOVA followed by a *post-hoc* Student-Newman-Keuls test. $P < 0.05$ was taken as the level of significance. Data are presented as means \pm s.e.mean for the indicated number of rats.

Results

Effects of ACTH₁₋₂₄ on plasma levels of ACTH and corticosterone

A dose-response curve for 0.03, 0.30 and 3.00 μg i.c.v. injected ACTH₁₋₂₄ was established for the activation of the HPA-axis. The two highest doses (0.3 and 3 μg) significantly increased the plasma concentrations of ACTH and corticosterone as

compared to saline (Figure 1). An intermediate active dose of 1 μg ACTH₁₋₂₄ was chosen for further experimentation.

The data in Figure 2 show that the increase of plasma ACTH and corticosterone levels following i.c.v. injection of 1 μg ACTH₁₋₂₄ was completely blocked by pretreatment of the rats with dexamethasone. Since this synthetic glucocorticoid is known to block the HPA-axis at the level of the pituitary and the brain (Keller-Wood & Dallman, 1984), this indicates that the increase in plasma ACTH and corticosterone observed after i.c.v. injection of ACTH₁₋₂₄ was not due to leakage of peptide from the ventricular system into the general circulation. Thus, in accord with our earlier observations (Wiegant *et al.*, 1979), we concluded that the effect resulted from an action of the peptide on targets in the brain.

Effects of SHU 9119 on ACTH₁₋₂₄-induced HPA-activation and excessive grooming

I.c.v. injections of 1 μg and 3 μg of the non-selective MC3-/MC4-R antagonist SHU 9119 alone, did not affect the plasma ACTH and corticosterone concentrations, and did not induce excessive grooming (Figure 3). ACTH₁₋₂₄ given i.c.v. at a dose of 1 μg significantly increased plasma ACTH and corticosterone concentrations and induced excessive grooming as compared to the saline-treated animals. Plasma ACTH and

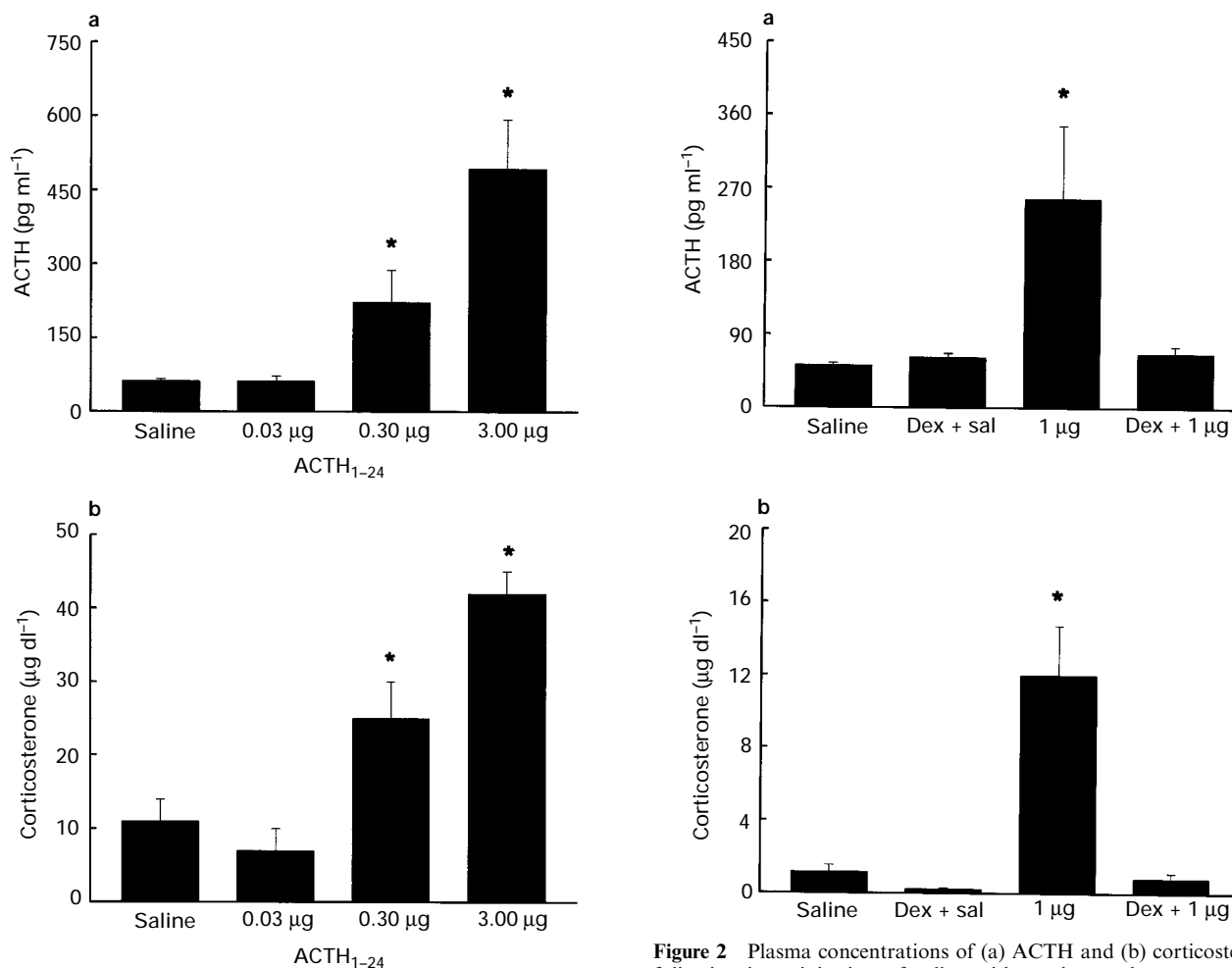


Figure 1 Plasma concentrations of (a) ACTH and (b) corticosterone following i.c.v. application of 0.03 μg ($n=8$), 0.30 μg ($n=8$) and 3.00 μg ($n=7$) ACTH₁₋₂₄ and saline ($n=16$). * $P < 0.05$ vs all other groups.

Figure 2 Plasma concentrations of (a) ACTH and (b) corticosterone following i.c.v. injection of saline without dexamethasone pretreatment (saline, $n=15$) or after dexamethasone pretreatment (Dex + sal, $n=7$), and of 1 μg ACTH₁₋₂₄ without dexamethasone pretreatment (1 μg , $n=6$) or after dexamethasone pretreatment (Dex + 1 μg , $n=6$). * $P < 0.05$ vs all other groups.

grooming responses following i.c.v. injection of 1 μg ACTH₁₋₂₄ were significantly antagonized by 1 and 3 μg SHU 9119. The plasma corticosterone response following i.c.v. injection of ACTH₁₋₂₄ was significantly antagonized by the 1 μg dose of SHU 9119, whereas the effect of 3 μg dose on the corticosterone response did not reach significance. No significant difference between the effect of 1 and 3 μg SHU 9119 in ACTH₁₋₂₄-treated rats was found. The 3 μg dose of SHU 9119, when coadministered with ACTH₁₋₂₄, caused barrel

rotation or other behavioural abnormalities in some animals (33%). These rats were eliminated from statistical analysis.

Effects of [D-Arg⁸]ACTH₄₋₁₀ on ACTH₁₋₂₄-induced HPA-activation and excessive grooming

[D-Arg⁸]ACTH₄₋₁₀ (i.c.v.) at doses of 5 μg and 10 μg , did not induce either a neuroendocrine or a grooming response (Figure 4). The increase of plasma ACTH and corticosterone

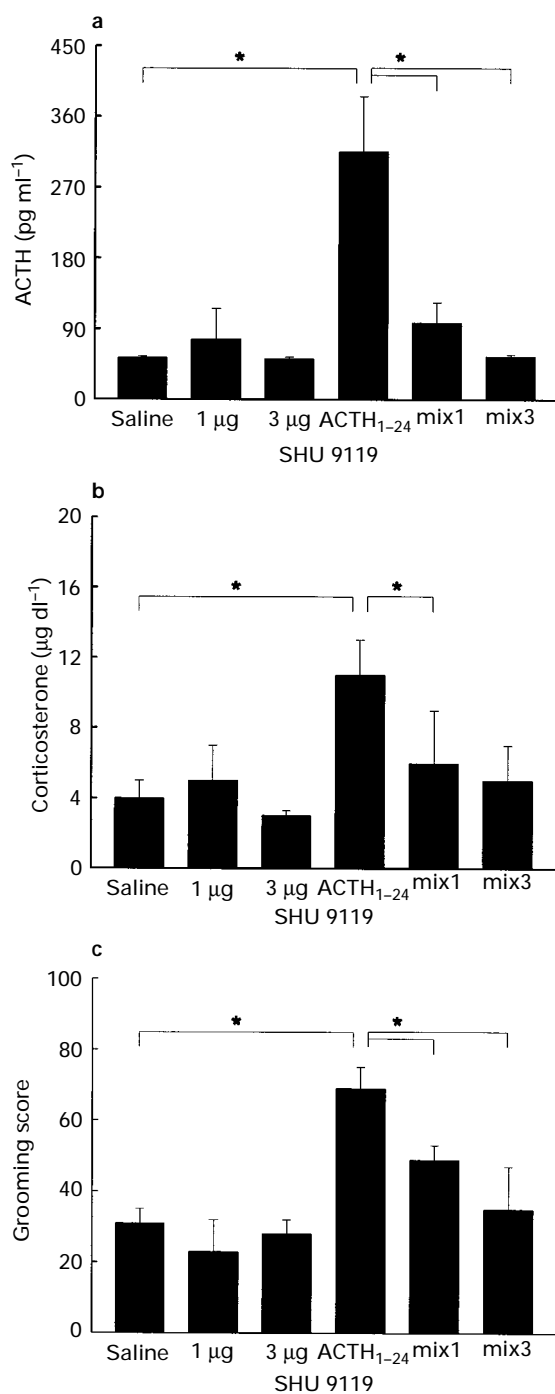


Figure 3 Plasma concentrations of (a) ACTH and (b) corticosterone and (c) grooming behaviour following i.c.v. injection of saline ($n=15$), 1 μg ($n=5$) and 3 μg ($n=6$) SHU 9119, 1 μg ACTH₁₋₂₄ ($n=11$), and after the administration of the mixture of 1 μg ACTH₁₋₂₄+1 μg SHU 9119 (mix1, $n=7$), and the mixture of 1 μg ACTH₁₋₂₄+3 μg SHU 9119 (mix3, $n=5$). * $P<0.05$.

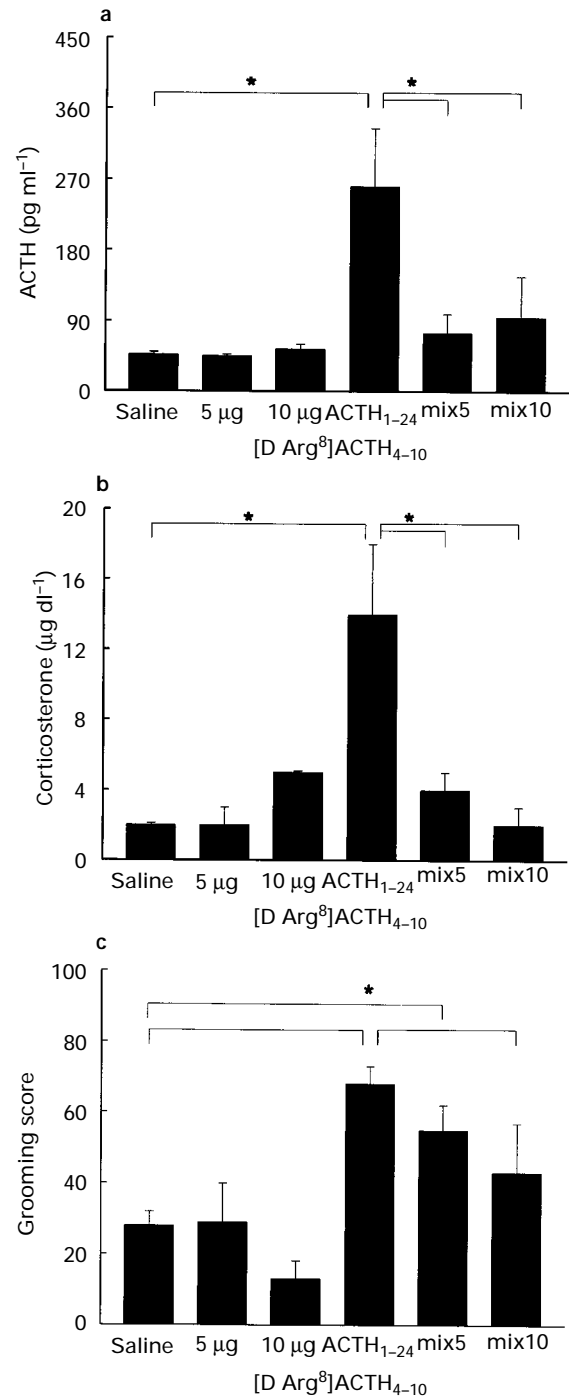


Figure 4 Plasma concentrations of (a) ACTH and (b) corticosterone and (c) grooming behaviour following i.c.v. injection of saline ($n=15$), 5 μg ($n=6$) and 10 μg ($n=6$) [D-Arg⁸]ACTH₄₋₁₀, 1 μg ACTH₁₋₂₄ ($n=12$), and after administration of the mixture of 1 μg ACTH₁₋₂₄+5 μg [D-Arg⁸]ACTH₄₋₁₀ (mix5, $n=6$), and the mixture of 1 μg ACTH₁₋₂₄+10 μg [D-Arg⁸]ACTH₄₋₁₀ (mix10, $n=5$). * $P<0.05$.

concentrations, and of grooming behaviour following i.c.v. injection of 1 µg ACTH₁₋₂₄ was significant as compared to the saline-treated animals. The ACTH₁₋₂₄-induced increase in plasma ACTH and corticosterone concentration was significantly inhibited by coadministration with 5 or 10 µg [D-Arg⁸]ACTH₄₋₁₀. The grooming response following i.c.v. injection of 1 µg ACTH₁₋₂₄ was significantly antagonized by the 10 µg dose of [D-Arg⁸]ACTH₄₋₁₀, whereas the inhibition of the grooming response by the 5 µg dose of the antagonist did not reach significance. No significant difference between the effect of 5 and 10 µg [D-Arg⁸]ACTH₄₋₁₀ in ACTH₁₋₂₄-treated rats was found for the grooming response.

Effects of Lys-γ₂-MSH on the HPA-axis and on excessive grooming

No significant effects of i.c.v. injection of 10, 20 and 50 µg doses of Lys-γ₂-MSH on plasma ACTH and corticosterone levels and grooming behaviour, were observed (data not shown).

Discussion

The present study confirms and extends our earlier observations that i.c.v. injection of ACTH stimulates the HPA-axis and induces excessive grooming behaviour, and that these effects arise from actions of the peptide within the central nervous system.

The present study further shows that the neuroendocrine, as well as the grooming responses induced by central administration of ACTH₁₋₂₄, can be inhibited by the non-selective antagonist for the MC₃- and MC₄-receptors, SHU 9119. A similar result was obtained with the selective MC₄-receptor antagonist, [D-Arg⁸]ACTH₄₋₁₀. These results indicate that the MC₄-receptor is involved in both effects of ACTH₁₋₂₄, but do not exclude a role for the MC₃-receptor. However, the selective MC₃-receptor agonist, Lys-γ₂-MSH, failed to elicit a neuroendocrine or a grooming response, suggesting that the MC₃-receptor is not involved. Furthermore, no agonistic activity of SHU 9119 was observed on the HPA-axis or grooming. Since SHU 9119 retains agonistic activity for the MC₃-receptor, this indicates that a role for the MC₅-receptor in these responses is unlikely. Taken together, the results therefore indicate that the activation of the HPA-axis by central administration of ACTH₁₋₂₄ is probably mediated by the MC₄-receptor, as has been shown previously for the induction of excessive grooming (Adan *et al.*, 1994b).

A role for the MC₄-receptor in the activation of the HPA-axis is in line with the distribution of MC₄-receptor mRNA in the brain. Firstly, MC₄-receptor mRNA is found in the dorsal medial parvocellular part of the paraventricular nucleus (PVN) of the hypothalamus (Mountjoy *et al.*, 1994), which gives rise to most of the CRH fibres that project to the external zone of the median eminence, and by that means is associated with the regulation of pituitary-adrenal activity (Swanson, 1986). Secondly, MC₄-receptor mRNA is found in several brain

areas that are associated with afferent control of the PVN, such as the bed nucleus of the stria terminalis, the subfornical organ, the parabrachial nucleus, the nucleus of the solitary tract, and other hypothalamic areas (Swanson, 1986; Mountjoy *et al.*, 1994).

The role of the MC₄-receptor in the excessive grooming response is supported by earlier findings, showing that: (i) MC₄-receptor antagonists inhibit the induction of excessive grooming behaviour induced by central administration of α-MSH (Adan *et al.*, 1994b), (ii) excessive grooming can be induced by local injection of ACTH₁₋₂₄ in the periaqueductal gray (Spruijt *et al.*, 1984), the periventricular nucleus (Van Erp *et al.*, 1991) and the substantia nigra (Wiegant *et al.*, 1977), areas where expression of the MC₄-receptor has been demonstrated (Mountjoy *et al.*, 1994). The absence of (i.c.v.) ACTH₁₋₂₄-induced excessive grooming in animals which are lesioned in the periaqueductal gray suggests a predominant role of the MC₄-receptor in this area in ACTH-induced grooming (Spruijt *et al.*, 1986) and (iii) the structure-activity relationships for excessive grooming induction by melanocortins resemble those for the induction of adenosine 3':5'-cyclic monophosphate (cyclic AMP) accumulation in HEK 293 cells expressing the MC₄-receptor (Adan *et al.*, 1994a), and in brain slices (Florijn *et al.*, 1993) of areas in which the MC₄-receptor has been found (Mountjoy *et al.*, 1994).

The observations that SHU 9119 blocked the excessive grooming response at both doses (1 and 3 µg), and that [D-Arg⁸]ACTH₄₋₁₀ was only effective at the 10 µg dose, might be explained by the fact that [D-Arg⁸]ACTH₄₋₁₀ is a less potent antagonist for the MC₄-receptor than SHU 9119, based on the pA₂-values found *in vitro* (Adan *et al.*, 1994b; Hruby *et al.*, 1995). However, the difference in effectiveness of these antagonists may also be caused by differences in pharmacokinetic properties.

In an earlier study from our laboratory (Wiegant *et al.*, 1979) a dissociation of the neuroendocrine and grooming responses to central administration of ACTH was found, and it was suggested that the two responses are brought about by two different interactions of ACTH and the brain, involving different neural substrates with possibly multiple receptors. In that study a reduced effectiveness of a second i.c.v. injection of ACTH in inducing the grooming response was found, whereas no single-dose tolerance was observed for the effect of i.c.v. ACTH on the HPA-axis. However, the present results clearly indicate that the rise in plasma concentrations of ACTH and corticosterone and the display of excessive grooming are mediated by the same melanocortin receptor type, the MC₄-receptor. Thus, the dissociation between the neuroendocrine and the grooming responses with regard to single dose tolerance is not dependent on the type of melanocortin receptor, but more likely on the localization and the connectivity of the target neurones involved.

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References

- ADAN, R.A.H., CONE, R.D., BURBACH, J.P.H. & GISPEN, W.H. (1994a). Differential effects of melanocortin peptides and neural melanocortin receptors. *Mol. Pharmacol.*, **46**, 1182–1190.
- ADAN, R.A.H., OOSTEROM, J., LUDVIGSDOTTIR, G., BRAKKEE, J.H., BURBACH, J.P.H. & GISPEN, W.H. (1994b). Identification of antagonists for melanocortin MC₃, MC₄ and MC₅ receptors. *Eur. J. Pharmacol.*, **269**, 331–337.
- BRAKKEE, J.H., WIEGANT, V.M. & GISPEN, W.H. (1979). A simple technique for rapid implantation of a permanent cannula into the rat brain ventricular system. *Lab. Animal Sci.*, **29**, 78–81.
- CHHAJLANI, V. & WIKBERG, J.E.S. (1992). Molecular cloning and expression of the human melanocyte stimulating hormone receptor cDNA. *FEBS Lett.*, **309**, 417–420.

- CHHAJLANI, V., MUCENIECE, R. & WIKBERG, J.E.S. (1993). Molecular cloning of a novel human melanocortin receptor. *Biochem. Biophys. Res. Commun.*, **195**, 866–873.
- DE WIED, D. & JOLLES, J. (1982). Neuropeptides derived from pro-opiomelanocortin: behavioral, physiological, and neurochemical effects. *Physiol. Rev.*, **62**, 976–1059.
- EBERLE, A.N. (1988). *The Melanotropins*. Basel: Karger.
- FLORIJN, W.J., MULDER, A.H., VERSTEEG, D.H.G. & GISPEN, W.H. (1993). Adrenocorticotropin/ α -melanocyte-stimulating hormone (ACTH/MSH)-like peptides modulate adenylate cyclase activity in rat brain slices: evidence for an ACTH/MSH receptor-coupled mechanism. *J. Neurochem.*, **60**, 2204–2211.
- GANTZ, I., KONDA, T., TASHIRO, T., SHIMOTO, Y., MIWA, H., MUNZERT, G., WATSON, S.J., DELVALLE, J. & YAMADA, T. (1993a). Molecular cloning of a novel melanocortin receptor. *J. Biol. Chem.*, **268**, 8246–8250.
- GANTZ, I., MIWA, H., KONDA, Y., SHOMOTO, Y., TASHIRO, T., WATSON, S.J., DELVALLE, J. & YAMADA, T. (1993b). Molecular cloning, expression, and gene localization of a fourth melanocortin receptor. *J. Biol. Chem.*, **268**, 15174–15179.
- GISPEN, W.H., WIEGANT, V.M., GREVEN, H.M. & DE WIED, D. (1975). The induction of excessive grooming in the rat by intraventricular application of peptides derived from ACTH: structure-activity studies. *Life Sci.*, **17**, 645–652.
- GISPEN, W.H. & ISAACSON, R.L. (1986). Excessive grooming in response to ACTH. *Neuropeptides Behavior*, **1**, 273–312.
- GRIFFON, N., MIGNON, V., FACCHINETTI, P., DIAZ, J., SCHWARTZ, J.C. & SOKOLOFF, P. (1994). Molecular cloning and characterization of the rat fifth melanocortin Receptor. *Biochem. Biophys. Res. Commun.*, **200**, 1007–1014.
- HRUBY, V.J., LU, D., SHARMA, S.D., CASTRUCCHI, A.L., KESTERSON, R.A., AL-OBEIDI, F.A., HADLEY, M.E. & CONE, R.D. (1995). Cyclic lactam alpha-melanotropin analogues of AcNle⁴cy⁵-clo[Asp⁵,D-Phe⁷,Lys¹⁰]-alpha-melanocyte-stimulating hormone-(4-10)-NH₂ with bulky aromatic acids at position 7 show high antagonist potency and selectivity at specific melanocortin receptors. *J. Med. Chem.*, **38**, 3454–3461.
- KELLER-WOOD, M.E. & DALLMAN, M.F. (1984). Corticosteroid inhibition of ACTH secretion. *Endocrinol. Rev.*, **5**, 1–24.
- LABBÉ, O., DESARNAUD, F., EGGERICKX, D., VASSART, G. & PARMENTIER, M. (1994). Molecular cloning of a mouse melanocortin 5 receptor gene widely expressed in peripheral tissues. *Biochemistry*, **33**, 4543–4549.
- LOW, M.J., SIMERLY, R.B. & CONE, R.D. (1994). Receptors for the melanocortin peptides in the central nervous system. *Curr. Opin. Endocrinol. Diab.*, **1**, 79–88.
- MOUNTJOY, K.G., ROBBINS, L.S., MORTRUD, M.T. & CONE, R.D. (1992). The cloning of a family of genes that encode the melanocortin receptors. *Science*, **257**, 1248–1251.
- MOUNTJOY, K.G., MORTRUD, M.T., LOW, M.J., SIMERLY, R.B. & CONE, R.D. (1994). Localization of the melanocortin-4-receptor (MC4-R) in neuroendocrine and anatomic control circuits in the brain. *Mol. Endocrinol.*, **8**, 1–11.
- ROSELLI-REHFUSS, L., MOUNTJOY, K.G., ROBBINS, L.S., MORTRUD, M.T., LOW, M.J., TATRO, J.B., ENTWISTLE, M.L., SIMERLY, R.B. & CONE, R.D. (1993). Identification of a receptor for γ -melanotropin and other proopioidmelanocortin peptides in the hypothalamus and limbic system. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 8856–8860.
- SPRUIJT, B.M., COOLS, A.R. & GISPEN, W.H. (1984). The neural substrate involved in excessive grooming. *Neurosc. Lett., Suppl.*, **18**, S362.
- SPRUIJT, B.M., COOLS, A.R. & GISPEN, W.H. (1986). The periaqueductal gray: a prerequisite for ACTH-induced excessive grooming. *Behav. Brain Res.*, **20**, 19–25.
- SWANSON, L.W. (1986). Organization of mammalian neuroendocrine system. In *Handbook of Physiology - The Nervous System*, Vol. 4, ed. Mountcastle, V.B., Bloom, F.E. & Geiger, S.R. pp. 317–363. Baltimore: Waverly.
- TATRO, J.B. (1990). Melanotropin receptors in the brain are differentially distributed and recognize both corticotropin and α -melanocyte stimulating hormone. *Brain Res.*, **536**, 124–132.
- TATRO, J.B. (1993). Brain receptors for central and peripheral melanotropins. *Ann. New York Acad. Sci.*, **680**, 621–625.
- TATRO, J.B. & ENTWISTLE, M.L. (1994). Heterogeneity of brain melanocortin receptors suggested by differential ligand binding in situ. *Brain Res.*, **635**, 148–158.
- VAN ERP, A.M.M., KRUK, M.R., WILLEKENS-BRAMER, D.C., BRESSER, W.M.A., ROELING, T.A.P., VEENING, J.G. & SPRUIJT, B.M. (1991). Grooming induced by intrahypothalamic injection of ACTH in the rat: comparison with grooming induced by intrahypothalamic electrical stimulation and i.c.v. injection of ACTH. *Brain Res.*, **538**, 203–210.
- VAN OERS, J.W.A.M. & TILDERS, F.J.H. (1991). Non-adrenocorticotropin mediated effects of endogenous corticotropin-releasing factor on the adrenocortical activity in rat. *J. Neuroendocrinol.*, **3**, 119–121.
- WIEGANT, V.M., COOLS, A.R. & GISPEN, W.H. (1977). ACTH-induced excessive grooming involves brain dopamine. *Eur. J. Pharmacol.*, **41**, 343–345.
- WIEGANT, V.M., JOLLES, J., COLBERN, D.L., ZIMMERMANN, E. & GISPEN, W.H. (1979). Intracerebroventricular ACTH activates the pituitary-adrenal system: dissociation from a behavioral response. *Life Sci.*, **25**, 1791–1796.

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